CLAIMS

What is claimed is:

1. A method of identifying a target nucleic acid sequence variation comprising;

a. providing a sample potentially containing one or more target polynucleotide;

b. providing one or more oligonucleotide probe sets, each set characterized by (i) a first oligonucleotide probe, having a first target-specific portion and a first barcode, and (ii) a second oligonucleotide probe, having a second target-specific portion and a second barcode, wherein the first oligonucleotide probe and the second oligonucleotide probe in a particular set are suitable for ligation together when hybridized adjacent to one another on a corresponding target polynucleotide;

c. providing a ligase,

d. blending the sample, the one or more oligonucleotide probe sets, and the ligase to form a mixture;

e. subjecting the mixture to one or more ligase detection reaction cycles comprising a hybridization treatment, a ligation step and a denaturation treatment, wherein the oligonucleotide probe sets hybridize at adjacent positions to form a ligated product containing the first barcode, the target-specific portions connected together, and the second barcode;

f. providing a solid support with one or more surface-bound probes on an array, wherein the surface-bound probes are complementary to the first barcode;

g. contacting the ligated product of step (e) with the solid support under conditions effective for hybridization of the first barcode with the surface-bound probes;

h. providing a third barcode carrying one or more detectable labels and a nanoparticle attached therein, wherein the third barcode is complementary to the second barcode; and

i. detecting the presence of the detectable labels on the ligated product captured on the solid support at particular sites, thereby detecting the nucleic acid sequence variation in the sample.

2. The method of Claim 1, wherein the target nucleic acid sequence variation is a single nucleotide polymorphism.

3. The method of Claim 1, wherein the surface-bound probes capture a normal target polynucleotide, a mutant target polynucleotide, or both.

4. The method of Claim 3, wherein the nanoparticle is attached at a 5' end or a 3' end of the third barcode.

5. The method of Claim 1, wherein the detectable labels comprise one or more dyes.

6. The method of Claim 5, wherein the one or more dyes have different surface-enhanced Raman spectra signatures

7. The method of Claim 5, wherein one or more dyes comprise cyanine dye, R110, R6, TAMRA, ROX, FAM, JOE, ZOE, TET, HEX, NAN, Texas Red, Rhodamine Red, Alexa dyes, or a combination thereof.

8. The method of Claim 7, wherein the cyanine dye comprises CYA, Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, or Cy7.5.3.

9. The method of Claim 1, wherein the mutant target polynucleotide differs from the target polynucleotide in the sample at one or more single nucleotide positions.

10. The method of Claim 1, wherein the nucleic acid sequence variations comprises multiple allele differences at a single nucleotide position.

11. The method of Claim 1, wherein the nucleic acid sequence variations comprise multiple allele differences at two or more nucleotide positions.

12. The method of Claim 1, wherein the nucleic acid sequence variations comprise multiple allele differences at nucleotide positions in multiple target polynucleotides.

13. A method of Claim 1, wherein the target-specific portions of the oligonucleotide probe sets have substantially the same melting temperature so that they hybridize to the target polynucleotides under similar hybridization conditions.

14. The method of Claim 1, wherein the nucleic acid sequence variations comprise insertions, deletions, microsatellite repeats, translocations, mutations, or a combination thereof.

15. The method of Claim 1, wherein the denaturation treatment is at a temperature of about 70 °C to about 105

16. The method of Claim 1, wherein the target-specific portions of the oligonucleotide probes each have a hybridization temperature of from about 40 °C to about 85 °C.

17. The method of Claim 16, wherein the hybridization temperature is from about 60 °C to about 70 °C.

18. The method of Claim 1, wherein the denaturation and the hybridization is from about 30 seconds to about 5 minutes long.

19. The method of Claim 1, wherein step (e) is repeated for about 2 to about 50 cycles.

20. The method of Claim 1, wherein step (e) takes about 1 to 250 minutes.

21. The method of Claim 1, wherein the ligase is selected from the group consisting of Thermus aquaticus ligase, Thermus thermophilus ligase, E. coli ligase, T4 DNA ligase, Thermus sp. AK16 ligase, Aquifex aeolicus ligase, Thermotoga maritima ligase, and Pyrococcus ligase.

22. The method of Claim 1, wherein the target-specific portions of the oligonucleotide probes are from about 15 to about 30 nucleotides long.

23. The method of Claim 1 further comprising, amplifying the target polynucleotides in the sample prior to the ligase.

24. The method of Claim 23, wherein the amplifying is carried out by subjecting the sample to a polymerase-based amplifying procedure.

25. The method of Claim 1, wherein the solid support is made from a material selected from the group consisting of plastic, ceramic, metal, resin, gel, glass, silicon, and composites thereof.

26. The method of Claim 1, further comprising treating the ligated product chemically or enzymatically after step (e) to remove unligated oligonucleotide probes.

27. The method of Claim 25, wherein the treating step is carried out with an exonuclease.

28. The method of Claim 1, wherein the target polynucleotide is a genomic DNA.

29. The method of Claim 1, wherein the ligated product is amplified with additional universal primers and DNA polymerase after ligation.

30. A method of identifying nucleic acid sequence variations in a sample comprising;

a. providing a sample potentially containing one or more target polynucleotides with at least one nucleotide variation;

b. providing one or more oligonucleotide probe sets, each set characterized by (i) a first oligonucleotide probe, having a first target-specific portion and a first barcode, (ii) a second oligonucleotide probe, having a second target-specific portion and a second barcode; and (iii) a third oligonucleotide probe, having a third target-specific portion and a third barcode, wherein the first target-specific portion in the first oligonucleotide probe in a particular set is suitable for ligation with the second target-specific portion in the second oligonucleotide probe, or the third target-specific portion in the third oligonucleotide probe;

c. providing a ligase;

d. blending the sample, the plurality of oligonucleotide probe sets, and the ligase to form a mixture;

e. subjecting the mixture to one or more ligase detection reaction cycles comprising a hybridization treatment and a denaturation treatment, wherein the oligonucleotide probe sets hybridize at adjacent positions to form at least two ligated product, the first ligated product containing the first barcode, the first target-specific portion connected with the second target-specific portion, and the second barcode, the second ligated product containing the first barcode, the first target-specific portion connected with the third target-specific portion, and the third barcode;

f. providing a solid support with a plurality of surface-bound probes on an array, wherein the surface-bound probes are complementary to the first barcode;

g. contacting the first and the second ligated product of step (e) with the solid support under conditions effective for hybridization of the first barcode with the surface-bound probes;

h. providing a fourth barcode carrying one or more detectable labels and a nanoparticle attached therein, wherein the fourth barcode is complementary to the second barcode;

i. providing a fifth barcode carrying one or more detectable labels and a nanoparticle attached therein, wherein the fifth barcode is complementary to the third barcode; and

j. detecting the presence of the detectable labels on the first ligated product, the second ligated product, or both on the solid support at a particular site, thereby indicating the presence of one or more nucleic acid variation in a sample.

31. The method of Claim 30, wherein the second and the third oligonucleotide probes capture allelic variants of a target polynucleotide.

32. The method of Claim 30, wherein the detectable label carried by the fourth barcode comprises a first dye and the detectable label carried by the fifth barcode comprises a second dye.

33. The method of Claim 32, wherein the first and second dyes have different surface-enhanced Raman spectra signatures.

34. The method of Claim 1, wherein the nucleic acid variations comprises a single nucleotide polymorphism.

35. The method of Claim 34, wherein the first and second dyes are selected from the group consisting of cyanine dyes, R110, R6, TAMRA, ROX, FAM, JOE, ZOE, TET, HEX, NAN, Texas Red, Rhodamine Red, or Alexa dye.

36. The method of Claim 35, wherein the cyanine dye comprises CYA, Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, or Cy7.5.

37. A diagnostic test kit for detection of nucleic acid variations in a sample comprising:

(a) a plurality of oligonucleotide probe sets, each set characterized by (i) a first oligonucleotide probe, having a target-specific portion and a first barcode, and (ii) a second oligonucleotide probe, having a target-specific portion and a second barcode, wherein the first oligonucleotide probe and the second oligonucleotide probes in a particular set are suitable for ligation together when hybridized adjacent to one another on a corresponding target polynucleotide, but have a mismatch which interferes with such ligation when hybridized to any other polynucleotide present in the sample; (b) ligase reagents; and (c) a third barcode carrying one or more detectable labels and a nanoparticle attached therein, wherein the third barcode is complementary to the second barcode.

38. The diagnostic test kit of Claim 37, wherein the third barcode comprises at least two different barcode sequences

39. The diagnostic test kit of Claim 37, wherein the nanoparticle and the third barcode are in separate containers, and the third barcode is attached to the nanoparticles prior to performing an assay.

40. The diagnostic test kit of Claim 37, wherein the nanoparticle, the third barcode, or both are functionalized prior to attachment of the nanoparticle.

41. The diagnostic test kit of Claim 37 further containing a substrate, the substrate having attached thereto a probe that hybridizes to the first barcode.

42. The diagnostic test kit of Claim 41, wherein the probe is a DNA microarray.